REMARKS

The Office Action of May 7, 2003 presents the examination of claims 2, 5 and 13, the remaining claims 8, 23 and 25-33 having been withdrawn from consideration. This paper makes a minor amendment to claim 8, to correct a problem of lack of antecedent basis.

Interview

An interview with the Examiner was held on August 12, 2004, and a written interview summary record is of record in the file. In the interview, it was agreed that claims 23 and 25-33 would be rejoined to the present application for examination. It was also agreed that the Examiner would consider a Declaration filed by Applicants attesting to unexpected results of the combination of the fluorophore described in the Toyo'oka reference with carbonic anhydrase.

It was also discussed during the interview that rejoinder of claim 8 should be considered, as the presently examined claims are directed to a composition comprising carbonic anhydrase and a photoluminescent molecule. Claim 8 is directed to the same, but having a particular arrangement of the carbonic anhydrase and the photoluminescent molecule in that the two components are covalently attached to one another.

The Examiner agreed in the interview to at least consider rejoining of claim 8 to the present application and such is again respectfully requested. Applicants submit that no undue burden of search is imposed upon the Examiner to examine claim 8 in the instant application, as any reference that teaches a carbonic anhydrase covalently joined to a photoluminescent molecule should be revealed by a search for either or both of these components separately.

Rejection for obviousness

Claims 2, 5 and 13 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Thompson '517 or Thompson et al., *J. Fluorescence* (1992), in view of Toyo'oka et al., *Anal. Chem.* (1984). This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Applicants have previously argued that the instant invention is not *prima facie* obvious over the cited references. In particular, Applicants argued that Toyo'oka et al. merely provides description of the 7-fluorobenz-2-oxa-1,3-diazole-4-sulfonamide:β-mercaptoethanol adduct fluorophore, but does not provide any teaching that suggests suitability of that fluorophore for use in the instant invention. Applicants pointed especially to the requirement for a fluorophore to have a short fluorescence lifetime, and the lack of such data in the

reference. Applicants further previously argued that, as such data were lacking from the Toyo'oka reference, the suitability of the fluorophore for use in the method disclosed in the instant application could be viewed as an unexpected result.

To these arguments, Applicants now add Declaration testimony attesting to aspects of the combination of the ABD-M fluorophore with carbonic anhydrase that would have been unexpected by the skilled artisan who read Toyo'oka and the cited references at the time the invention was made. Applicants submit that these aspects evidence patentability of the presently claimed invention over the cited prior art.

The attached Thompson Declaration establishes that the usual result of binding of a fluorophore to a protein is quenching of any fluorescence emission, typically mediated by electron transfer from tryptophan residues present in most proteins. On the other hand, binding of the ABD-M fluorophore to carbonic anhydrase results in an unexpected <u>increase</u> in fluorescence and also to an unexpected blue shift in the fluorescence, sufficient to one to distinguish a signal from free fluorophore from a signal from bound fluorophore. It was also found ABD-M binds to carbonic anhydrase sufficiently tightly that the fluorophore can be used at low enough concentration to be useful for measuring low amounts of divalent metal ions in a sample. Finally, Dr. Thompson points out that

ABD-M bound to carbonic anhydrase unexpectedly has a fluorescence lifetime short enough to make the fluorophore useful in methods that depend upon rotational correlation time measurements of free versus protein-bound fluorophores. None of these four properties could be expected by a skilled artisan reading the Thompson and Toyo'oka references. Accordingly, the instant invention is shown to be unobvious over these references.

For all of the above reasons, the rejection of claims 2, 3, and 13 under 35 USC § 103(a), over Thompson '517 or Thompson et al., *J. Fluorescence* (1992) in view of Toyo'oka, should be withdrawn. Such favorable action is respectfully requested.

Applicants submit that the present application well describes and claims patentable subject matter. The favorable action of withdrawal of the standing rejections and allowance of the application is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mark J. Nuell (Reg. No. 36,623) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), Applicants respectfully petition for two (2) months extension of time for filing a response in connection with the present application. The required fee of $215.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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3645-0104P

Attachment: Thompson Declaration with his Curriculum Vitae
Exhibits 1-3
Journal of Biological Chemistry, Vol. 242, No. 24 (December 25, 1967), pp. 5813-5823